REMARKS

Claims 1-15 and 31-32 were pending in the subject application. By this amendment, Claims 1, 3, 4, 13, 14 and 32 have been amended. Support for amendments to Claims 1, 3, 4, 13 and 14 can be found in the specification at least on page 14, lines 16-27 of the application as filed. Further support for the amendments to Claim 1 can be found at least on page 6, lines 1-6, page 7, lines 24-30, page 11, lines 11-13, and page 12, line 27 through page 13, line 12 of the application as filed. Support for the amendment to Claim 32 can be found in the previous version of the claim.

The specification has been amended to provide an Abstract on a separate sheet. Support for the Abstract can be found in the Abstract for PCT/EP03/06049 (WO 03/105058), which was filed as part of the subject application.

Applicants maintain that the amendments to the specification and to the claims do not represent an issue of new matter. Accordingly, entry of the amendments is respectfully requested.

Abstract

The Examiner objected that an Abstract has not been provided on a separate sheet of paper. In reply, applicants submit herewith an Abstract on a separate sheet of paper.

Rejections under 35 U.S.C. §112, Second Paragraph

A. Claim 1, step b): The Examiner indicated that the phrase "obtaining ... representations of peptide backbone structures of ... peptide... located within the binding site of said MHC molecule" is not clear.

In reply, step b) has been amended to recite:

"obtaining an ensemble of conformational representations of peptide backbone structures of said peptide, said conformational representations located within the binding site of said MHC molecule..."

The functional characteristics of the subject-matter of claim 1 can be highlighted as follows:

(1) A representation of a MHC class I or class II molecule is provided.

As known in the art and documented in the description (*e.g.* paragraph spanning pages 2-3 of application as filed), each MHC molecule has exactly one peptide binding site (also known as the binding groove).

(2) Representations of peptide backbone structures (together forming an ensemble) are obtained.

As known in the art and documented in the description (*e.g.* paragraph spanning pages 2-3 of application as filed), any peptide being bound to a MHC binding site is conformationally restricted by the MHC molecule.

Although a MHC-bound peptide is to a certain extent conformationally restricted, both the binding site of the MHC and the peptide have a somewhat flexible structure. The instant method explicitly exploits this feature by providing an ensemble of different peptide backbone structures, rather than a single, fixed structure as most methods do. Step b) of Claim 1 simply states that a plurality (ensemble) of slightly different peptide backbone conformations are placed in the MHC binding site, which effectively renders the representations dependent on the MHC molecule. Even though the peptides can adopt various conformations, they are effectively limited to fit in the MHC binding site. As such, any specific conformation does depend on the presence of other molecules, *i.e.* the MHC molecule.

To clarify the term "representations" in part b) of Claim 1, this term has been amended to recite "<u>conformational</u> representations" (as well as in Claims 3, 4, 13 and 14). Such amendment further highlights the relative flexibility, as a consequence of which different conformations can be adopted *within* the MHC binding site. Basis for this amendment can be found throughout the application (*e.g.* page 14, lines 16-27 of the application as filed).

B. Claim 1, step c): The Examiner requested clarification of what is modeled.

In reply, step c) has been amended to recite:

"modeling the side-chains of at least said peptide for each peptide backbone structure of said ensemble in relation to said MHC molecule, thereby obtaining an ensemble of modeled MHC/peptide complexes..."

This amendment highlights that (i) the side chains are modeled and (ii) in addition to the side chains of the peptide, also side chains of the MHC molecule can be modeled. Support for this amendment can be found *e.g.* on page 7, lines 24-30, and page 11, lines 11-13 of the application as filed.

C. Claim 1, step d2): The Examiner requested clarification of what constitutes "the conformational energy for the complete ensemble." The Examiner also requested clarification of which "ensemble" is meant.

In reply, step d2) has been amended to recite:

"evaluating the conformational entropy for the complete ensemble of step c)..."

Applicants note that Claim 1, step d2) does not refer to conformational "energy" as suggested by the Examiner but rather to conformational "entropy." This is an important distinction. Entropy is fundamentally a property of an ensemble, whereas conformational energy is tied to individual structures.

It is clearly stated in Claim 1 that individual conformational energies (one per complex of the ensemble, see step d1: "of each complex of the ensemble") and the conformational entropy of the ensemble as a whole (see step d2: "for the complete ensemble") are evaluated. See also page 8, lines 16-25, and page 11, lines 23-27 of the application as filed regarding the evaluation and scoring of energy and entropy.

A specific method to derive binding affinities from these contributions is provided in Example 4; on page 17, line 21 through page 19, line 19; and on page 25, line 24 through page 28, line 19 of the application as filed.

Regarding which ensemble is meant in step d), applicants note that there really is only one ensemble, which is gradually built up, as follows: There is only one ensemble, albeit that the ensemble in step b) is still incomplete after execution of step b) because the side chains are yet to be added in step c). Applicants remark that the ensemble is gradually built up in steps a), b) and c), and is finally evaluated in step d).

Reconsideration and withdrawal of these rejections are respectfully requested.

Rejections under 35 U.S.C. §101

Claims 1-15 and 31-32 are rejected because the claims do not recite the use of a particular machine or do not recite a physical transformation. In addition, the Examiner noted that the claims do not recite a tie to another category of invention.

In reply, Claim 1 has been amended to recite "the method being executed on a computer under the control of a program stored on the computer..." thus identifying the apparatus that accomplishes the method steps. In addition, the new step of Claim 1 of "outputting said evaluation to a user in a user-readable format" requires a physical transformation.

Support for the amendments can be found on page 12, line 27 through page 13, line 12 of the application as filed.

Reconsideration and withdrawal of this rejection is respectfully requested.

Rejections under 35 U.S.C. §103(a)

Claims 1-15 and 31-32 are rejected as being unpatentable over Fothergill et al. (WO98/59244) ("Fothergill"), in view of Background prior art addressed in the

specification, Rognan et al. (J. Med. Chem. 42: 4650-4658, 1999) ("Rognan"), Bohm (J. Computer-Aided Molecular Design 8: 243-56, 1994), Miyazawa et al. (Proteins: Structure, Function, and Genetics 36: 357-369, 1999) ("Miyazawa"), and Gohlke et al. (Current Opinion in Structural Biology 11: 231-5, 2001) ("Gohlke").

Applicants respectfully traverse this rejection.

The present invention

The present invention relates to a structure-based method for predicting binding affinity of a peptide for a MHC molecule in which:

(1) an ensemble of MHC/peptide complexes is modeled by:

(a) providing an ensemble of conformational representations of peptide backbones;

(b) in addition, modeling of the peptide side-chains; and

(2) the binding properties of the peptide/MHC complexes are evaluated by:

  (a) evaluating the potential energy of each complex of the ensemble;

  (b) evaluating the conformational entropy of the complete ensemble.

Key issues of the present invention are thus:

(i) a structure-based binding prediction method;

(ii) an ensemble of peptide/MHC modeled conformational representations; and

(iii) scoring the binding affinity based on the combination of potential energy for each complex and the conformational entropy for the whole ensemble.

The use of structure-based binding prediction methods is well-known in the art (see *e.g.* paragraph spanning pages 4-5 of the application as filed). However, all are fundamentally different from the present invention in that:

(1) they rely on statistics (*i.e.* scoring based on probability or average effects), see *e.g.* Fothergill (WO 98/59244), cited in the present application;

(2) they rely on approximation (*i.e.* empirical and heuristic), see *e.g.* Rognan cited in the application and Miyazawa;

(3) they rely on well-documented (experimentally determined) data, see *e.g.* Bohm.

In general, such statistical and empirical methods yield poor correlation with experiment, especially when experimentally determined data are scarce (see *e.g.* Logean *et al.* (2001) on page 5 of the application as filed.

A major improvement of the present invention over the prior art is the recognition of the importance of determining the conformational entropy for **a whole ensemble of conformational complexes** in accurately assessing the binding affinity. In fact, the evaluation of the conformational entropy of the ensemble as a whole has never been demonstrated in the field, all the more because of the sheer complexity of the MHC receptors and peptidic ligands.

On page 6 of the Office Action, the Examiner set forth a characterization of the present invention. Applicants would like to highlight (**in bold type**) the lack of critical elements in the Examiner's assertions regarding the present invention, in particular Claim 1, relating to:

(a) receiving a representation of three-dimensional structure of a MHC class I or class II molecule;

(b) obtaining an ensemble of representations of peptide backbone structures **that are located within the binding site of said MHC molecule**;

(c) modeling side chains for the backbone structures to obtain an ensemble of MHC/peptide complexes **for each peptide**; and

(d) evaluating the binding properties of said peptide for said MHC molecule, by

d1) evaluating the potential energy **for each complex of the ensemble**; and

d2) evaluating the conformational entropy **for the complete ensemble**.

Fothergill

In respect of the evaluation of the **potential energy** (claim 1, step d1), Fothergill evaluates a conformational **score** and not a conformational **energy**. These

are two fundamentally different parameters. Conformational energies are typically calculated on basis of an energy function (commonly denoted "force field function", see page 25, top paragraph, of the application as filed). Such calculations are computationally very demanding, which is one of the reasons why most researchers switch to the alternative of statistical processing, in simple words: counting favorable and unfavorable contacts. This is exactly what Fothergill is doing: the complexes are checked for steric overlaps ("value B"), number of H-bonds are counted ("value C"), electrostatic interactions are considered ("value D") and the number of favorable contacts are counted ("value E"). Thus, **Fothergill teaches a simple statistical function, and not an energy function**, to evaluate the conformational "fitness" of MHC-bound peptides. This is understandable in view of the complexity of the task: a large MHC structure comprising a plurality of peptide conformations each of which have to be modeled in the context of the binding site. It is one of the key elements of the present invention that such can be accomplished by evaluating a detailed energy function.

In respect of the evaluation of **conformational entropy** (claim 1, step d2), the method by **Fothergill does not comprise any evaluation step of conformational entropy**. The latter is a second key element of the present invention. While already the evaluation of conformational energy is non-obvious for multiple MHC complexes, evaluation of conformational entropy is far more complicated. There are many highly technical reasons (and controversy in the literature) why the entropy of a conformational ensemble is rarely considered to evaluate complexes, and never considered to evaluate MHC/peptide complexes, despite the fact that it is an undeniable and fundamental component of free energy ($\Delta G = \Delta H - T\Delta S$). Applicants believe that this is because it is quite useless to compute ΔS (entropy) terms if ΔH ($\sim$energy) terms are not extremely accurate. Nevertheless, irrespective of this conviction, it is a fact that MHC/peptide complexes have *never* been evaluated while evaluating conformational

entropy of the ensemble. (Note, in this respect, that ensemble entropy is fundamentally distinct from the entropic loss caused by rotatable bond freezing, as described by Rognan, see below).

Rognan

The Examiner states that "the FRESNO function accounts both for the potential energy and entropy of a complex". Applicants respectfully disagree: the **entropic contributions calculated by Rognan does not correspond to the ensemble entropy**. Rognan (p. 4656, right column, relating to Eq. 10) states: "The rotational term (ROT) estimates the loss of entropy due to the freezing of rotatable bonds of the ligand upon binding". Applicants stress that the freezing of rotational bonds is an event that occurs locally in each individual complex, and is not to be confused with the entropy for a complete ensemble (*i.e.*, a single entropy value for a set of multiple and full MHC/peptide complexes) as in step d2) of the method of Claim 1.

Bohm

The Examiner states that "Bohm et al teaches scoring function that estimates the free energy of binding for a given protein-ligand complex of known 3D structure" and "The Bohm function accounts for both potential energy and entropy of the complex". Further, the "LUDI" function takes into account similar energy-like contributions as the FRESNO function: H-bonds, ionic interactions, lipophilic contact surface and number of rotatable bonds.

Applicants submit that:

(1) Bohm evaluates only complexes of already **known 3D structure**. As such, Bohm does not teach modeling of a plurality (ensemble) of peptide backbone structures within a MHC binding site. Also Bohm does not teach modeling of side chains on said peptide backbone structures. Further, Bohm does **not work with structural**

ensembles, but with single, fixed complexes of known (experimentally determined) 3D structure.

(2) Bohm evaluates binding free energies using a **parameterized scoring function** (see p. 244). Parameters are trained (calibrated, optimized) using a large set of protein-ligand complexes of known affinity and known three-dimensional structure (p. 246, TABLE 1). Different sets of optimized parameters are listed at p. 248, TABLE 2. Importantly, when the author applies the scoring function to complexes of known structure but unknown affinity, then only the potential energy terms (H-bonds, ionic interaction, lipophilic interactions and rotational terms) are evaluated; the term ΔG_0 itself is not evaluated, but just set equal to a constant. See Abstract: "The function also contains a constant contribution..."; p. 245, 2nd paragraph: "ΔG_0 ... does not directly depend on any specific interactions..."; p. 252, 2nd paragraph: "Function #2 contains a constant contribution ΔG_0 amounting to +5.4 kJ/mol."

(3) In the Discussion, at p. 252, 2nd paragraph, the author states that the constant term ΔG_0 may be interpreted as accounting for a loss of entropy upon binding, but at the same time cautions about its physical interpretation. Applicants submit that the ΔG_0 term is formally and technically just an offset in the parameterization procedure (to obtain a better fit to the data). Whether or not such term can be interpreted as an entropy term is a matter of debate.

In conclusion, the ΔG_0 term used by Bohm

(i) is subject to dubious interpretation;

(ii) is not calculated for each complex;

(iii) is applied to complexes of known structure but not to complexes wherein the ligand is constructed in the binding site (as in steps b and c of Claim 1);

(iv) is applied to fixed structures but not to complete ensembles (as in step d of Claim 1).

Consequently, Bohm does not teach the actions as specified in Claim 1, step d2) of the instant invention.

Miyazawa

The Fxaminer states that "Miyazawa et al. teach scoring function that estimates the **free energy of binding for a given protein-ligand complex** and evaluates stability of a specific conformation determined ... **relative to the whole ensemble of protein conformations.**" (Emphases added.)

Applicants respectfully submit that the Examiner is mistaken in his assertion that the scoring function of Miyazawa would estimate the free energy of binding: their scoring function is not used to estimate protein-ligand binding strength but to evaluate protein stability (see *e.g.* Abstract, 1st sentence). The method of the cited document thus relates to a different application (protein stability prediction, fold recognition and sequence recognition) than that of the instant application (MHC-peptide affinity).

In addition, the Examiner is correct in stating that Miyazawa determines the stability of a specific conformation relative to that of the whole ensemble of protein conformations (reference to equation 7 and its explanation is made). However, the act of determining the stability of a specific conformation relative to that of the whole ensemble (as performed by Miyazawa) is completely dissimilar to the act of evaluating the conformational entropy **of** an ensemble (as performed in the instant application). The former is about telling how much better one conformation scores relative to the whole ensemble (please note that there is no entropy in this), whereas the latter is about obtaining a measure of conformational entropy for the ensemble itself.

The Examiner then continues by "The estimate combines the free conformational energy and conformational entropy..." and mentions the usage of σ (sigma) as a constant to represent the conformational entropy per residue for native-like structures, referring to equation 10. Applicants respond to this that the conformational entropy per residue is

in no way related to the conformational entropy of an ensemble. Applicants refer to Miyazawa p. 360, last line to p. 361, 2nd line, stating that σ "is taken to be independent of residue type and is related to the deletion penalty parameter in sequence-structure alignments." Applicants maintain that the usage of a term reflecting deletion penalties in sequence-structure alignments would not teach, instruct or suggest the evaluation of conformational entropy term for a complete ensemble of MHC/peptide complexes.

Gohlke

The Examiner refers to Gohlke who reviewed a variety of scoring functions to evaluate protein-ligand binding. Indeed, many of the "Regression-based" and "First-principle-based" approaches include a variety of energetic and entropic contributions in their scoring functions.

Applicants welcome citation of this review article for it clearly demonstrates that the combination of conformational energy and entropy is not obvious. Applicants refer in this respect to the many studies by independent researchers who found that in their hands the entropic contributions were of "negligible influence", "showed very high fluctuations", "contributed little to the total binding energy", were "discarded from the final equation", etc. (see Gohlke, page 233, 2nd and 4th paragraphs). Applicants further submit that these problems could only be solved in the way as formulated in the instant claims. Support for the claimed subject matter and, more specifically, for the combination of conformational energy with ensemble entropy, is provided in the Example 4 of the present application (see Equation 7 and following discussion).

Applicants further submit that none of the references cited by the Examiner or cited in Gohlke suggest a method as claimed in the instant application. The only method bearing some functional similarity to that of the instant application is the "FRESNO" method of Rognan. These authors are the only ones who evaluate a plurality (ensemble) of peptide backbone conformations in a MHC binding groove. But even these experts failed to take into account the global entropy for the full ensemble of modeled MHC/peptide complexes. Instead, Rognan computed local entropy contributions accounting for the freezing of individual rotatable bonds, and this for each individual MHC/peptide complex (thus, ending up with one entropy value per complex, and not one entropy value per ensemble of complexes).

Conclusion

Applicants submit that the step of evaluating the entropy of a complete ensemble is not merely "a combination of known elements", or "a simple substitution of one known scoring method for another" or "a mere application of a known technique to a piece of prior art ready for improvement", as suggested by the Examiner.

Applicants maintain that the Examiner's reference to Miyazawa is of no relevance in this respect, because the cited passage relates to different technical handlings (the calculation of a partition function) and a different technical effect (the establishing of a reference state). The latter is not even related to the next cited passage at p. 10, lines 3-6, dealing with the calculation of σ, a measure of conformational entropy per residue (*i.e.*, a local contribution essentially reflecting a deletion penalty in sequence-structure alignments). Clearly, the cited passages relate to two different issues, and do not suggest any of the steps as specified in the claims of the instant invention.

In fact, none of the cited prior art documents suggests as a component of the prediction algorithm the determination of the conformational entropy of the whole ensemble of conformational peptide/MHC representations. At best the prior art teaches

that contribution of an entropy component (for **single** representations) is negligible or can be approximated by a constant value.

The present inventors, however, realized that accurate determination of the potential energy (and not approximation, estimation, ... via empirical or statistical methods) necessitates the determination of conformational entropy for the ensemble of conformational representations **as a whole**.

Applicants maintain that the claimed invention is not obvious over the cited art. Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

<u>Status of European Patent Family Member</u>

Applicants would like to direct the Examiner's attention to European Patent EP 1 516 275, attached hereto, which was granted with claims directed to the same subject matter as the present application.

CONCLUSIONS

In view of the preceding amendments and remarks, applicants respectfully request that the Examiner reconsider and withdraw the objection and rejections in the February 18, 2009 Office Action, and earnestly solicit allowance of the claims under examination. If there are any minor matters preventing the allowance of the subject application, the Examiner is requested to telephone the undersigned attorney.

A check for $65.00 is enclosed for the fee for a one month extension of time for a small entity. No other fee is deemed necessary in connection with the filing of this Amendment. However, if any other fee is required with this reply or to maintain the pendency of the subject application, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 01-1785.

Respectfully submitted,

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By ______________________
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